REMARKS

In view of the preceding amendments and the comments which follow, and pursuant to 37 CFR §1.111, amendment and reconsideration of the Official Action of April 19, 2004 is respectfully requested by Applicants.

A claims listing and specification pages marked to show changes are submitted herewith.

The specification has been amended to insert missing serial numbers and to delete attorney docket numbers.

Claims 2-3, 5-6, 9-12, 14-15, 17-18, 22-25, 27-28, and 31-32 have been canceled without prejudice. Claims 1, 16, 29, and 30 have been amended. Support for recitations added are found throughout the originally filed application. No new matter has been added.

Claims 1, 4, 7-8, 13, 16, 19-21, 26, 29-30, and 33-34 remain pending for examination.

Amendment to specification

The Examiner has noted that the application serial numbers missing on pages 1, 11, and 16 of the specification must be supplied. Applicants have complied by way of the present amendment.

Rejection under 35 USC §101

Claims 1-34 have been rejected under 35 USC §101 as claiming the same invention as that of claims 1-34 of prior US application 10/053,058.

Applicants note for the Examiner's information that US application 10/053,058 has now been abandoned. A Notice of Express Abandonment was filed by Applicants on 4/28/04. Reconsideration of the rejection is requested.

Rejection under 35 USC §112, first paragraph

Claims 1-15 have been rejected under 35 USC §112, first paragraph, because the specification, while being enabling for "treating said sensitized particles in an aqueous mixture with an amine compound" wherein the treatment results in the reaction of the "succinimide ester groups" with the amine functionality of the "amine compound" to form an amide linkage as set forth at page 9, lines 13-17 of the specification, does not reasonably provide enablement for "treating" wherein no such reaction occurs.

Applicants have amended claim 1 (and thereby claims 4, 7-8, and 13 depending therefrom) to recite "wherein the treatment results in the reaction of the succinimide ester groups with the amine functionality of the amine compound to form an amide linkage". The arguments for rejection having thus been avoided or overcome, Applicants request the Examiner's reconsideration of the rejection.

Rejection under 35 USC §112, first paragraph

Claims 16-34 have been rejected under 35 USC §112, first paragraph, because the specification, while being enabling for the preparation of a "sensitized particle" wherein the "at least one antibody" is bound to the surface by reaction of the NHS/CDI-activated carboxylate groups on the particle surface with an amine group on the antibody (claims 16, 29, and 30), does not reasonably provide enablement for an antibody bound to the surface through any/all types of "covalent bond". The invention described in the specification is directed specifically to NHS/CDI-activated carboxylate groups which are used to attach both the antibody and the amine compound to the particles. Further, the Examiner remarks that the specification is also enabling only for the case in which the "succinimide ester" is covalently attached to the particle surface. The claim 16 term "the

reaction product of a succinimide ester and an amine compound of formula (I) on the surface" does not require such an attachment; this term includes the case wherein the "reaction product" is simply coated on the surface.

Applicants have amended claims 16, 29, and 30 (and thereby claims 19-21, 26, and 33-34 depending therefrom) to recite a covalent bond "from the reaction of an N-hydroxysuccinimide or N-hydroxysulfosuccinimide/carbodiimide-activated carboxylate group on the particle surface with an amine group on the antibody". In addition, the claims have been amended by reciting that the reaction product is covalently attached to the particle surface. The arguments for rejection having thus been avoided or overcome, Applicants request the Examiner's reconsideration of the rejection.

Rejection under 35 USC §103 (a)

Claims 1-34 have been rejected under 35 USC §103 (a) as being unpatentable over each of Dijksma et al., *Anal. Chem. 73*:5, 901-907, 2001 (hereinafter "Dijksma") or Inzana, *J. Clin. Microbiol. 33*, 2297-2303, 1995 (hereinafter "Inzana"). The Examiner argues that the references describe a conventional method of attaching a ligand (antibody) to a particle utilizing a carbodiimide/succinimide (CDI/NHS) activation of carboxylate groups on the particle, the same method described in instant claim 1. The references describe the further addition of ethanolamine to deactivate residual succinimide groups on the particle surface. The structure of the succinimide deactivator ethanolamine (prior art) corresponds to the structure of the succinimide deactivator of formula (I) of instant claim 1 wherein R=2 and X=OH. However, the proviso of instant claim 1 limits R to the "range of 4 to 20 carbon atoms". It is the Examiner's position that, given the teachings of the prior art, i.e., that ethanolamine is a known deactivating agent for residual succinimido groups present after CDI/NHS coupling of a ligand to a carboxylated particle, it would be obvious to substitute other well known homologs of ethanolamine [HO-$(CH_2)_2$-NH_2], for example HO-$(CH_2)_4$-NH_2, in the method of either Dijksma or Inzana, as claimed, with the expectation of obtaining a similarly useful succinimido

deactivation method which produces particles useful in immunoassays. The Examiner also argues that the features of the dependent claims are either specifically described by the references or constitute obvious variations in parameters which are routinely modified in the art and which have not been described as critical to the practice of the invention. Further, the packaging of reagents in kit form is an obvious expedient for ease and convenience in assay performance.

Applicants' claims now specifically recite that the amine compound is selected from the group consisting of 2,2'-(ethylenedioxy)bisethylamine and 4,7,10-trioxa-1,3-tridecanediamine. These two compounds are taught in the specification as particularly preferred (see page 9, lines 11-12). These compounds feature a "bidentate" character as described on page 9, lines 18-26. This bidentate character, i.e., where $X=NH_2$ in formula (I), is not present in compounds taught by the prior art, i.e., ethanolamine compounds where X=OH in formula (I). Applicants' discovery of markedly superior results when using these bidentate compounds was surprising. See Table 2 on page 22 that shows the marked superiority with regard to the amount of serum proteins bound in comparison with ethanolamine derivatives.

Applicants argue that neither Dijksma nor Inzana teach or suggest, nor do they provide the motivation to try, the bidentate compounds of Applicants' invention. The case for obviousness has not been made, and Applicants respectfully request the Examiner's reconsideration of the rejection of claims 1-34 under 35 USC §103 (a).

Rejection under 35 USC §103 (a)

Claims 1-34 have been rejected under 35 USC §103 (a) as being unpatentable over Dade Behring application WO 98/36277 (hereinafter "Dade") taken in combination with the admitted prior art as set forth at page 1, line 27, through page 2, line 17, of the instant specification.The Examiner argues that the instant specification establishes that CDI/NHS (succinimide) mediated linkage of antibodies to carboxylated particles is well

known in the art and that it is also well known that residual succinimide (NHS) esters remain on the particles after the reaction. Dade establishes that compounds of the type recited in instant claims 1, formula (I) wherein S=OH and R=alkyl ether are well known stabilizers for immunoassay particles which contain succinimidy (NHS) ester groups. It is the Examiner's position that, given the fact that amine- and hydroxy-substituted alkyl ethers are well known stabilizers for particles containing residual succinimidy ester groups, it would be obvious to use these stabilizers for particles containing the same residual succinimidyl ester groups present as a byproduct of using conventional NHS/CDI linking methodology, as claimed. The Examiner also argues that the features of the dependent claims are either specifically described by the references or constitute obvious variations in parameters which are routinely modified in the art and which have not been described as critical to the practice of the invention. Further, the packaging of reagents in kit form is an obvious expedient for ease and convenience in assay performance.

Applicants' claims now specifically recite that the amine compound is selected from the group consisting of 2,2'-(ethylenedioxy)bisethylamine and 4,7,10-trioxa-1,3-tridecanediamine. These two compounds are taught in the specification as particularly preferred (see page 9, lines 11-12). These compounds feature a "bidentate" character as described on page 9, lines 18-26. This bidentate character, i.e., where X=NH_2 in formula (I), is not present in compounds taught by the prior art, i.e., in ethanolamine compounds where X=OH in formula (I). Applicants' discovery of markedly superior results when using these bidentate compounds was unexpected and surprising. See Table 2 on page 22 that shows the marked superiority with regard to the amount of serum proteins bound in comparison with ethanolamine derivatives.

Applicants argue that the molecular surface modifiers taught by Dade are, as in Dijksma and Inzana, ethanolamine derivatives, i.e., compounds having a single amino functionality. The bidentate compounds of Applicants invention are neither taught nor suggested by Dade or by the prior art described in Applicants' specification. There is no motivation to attempt compounds having an amine functionality at both ends of the

molecule since Dade expressly teaches that R is H, CH_3, or CH_2CH_3. The case for obviousness has not been made, and Applicants respectfully request the Examiner's reconsideration of the rejection of claims 1-34 under 35 USC §103 (a).

* * * * * *

Applicants submit that their application is now in condition for allowance, and favorable reconsideration of their application in light of the above amendments and remarks is respectfully requested. Allowance of claims 1, 4, 7-8, 13, 16, 19-21, 26, 29-30, and 33-34 at an early date is earnestly solicited.

* * * * * *

The Examiner is hereby authorized to charge any fees associated with this Amendment to Deposit Account No. 02-2958. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

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CLAIMS LISTING 7/30/04

What is claimed is:

1. (currently amended) A method of preparing particles for immunoassays, comprising:

reacting particles comprising carboxylate groups with N-hydroxysuccinimide or N-hydroxysulfosuccinimide and with a carbodiimide coupling reagent to provide activated particles comprising succinimide ester groups;

contacting said activated particles with antibodies to provide sensitized particles comprising covalently bound antibodies and residual succinimide esters; and

treating said sensitized particles in an aqueous mixture with an amine compound selected from the group consisting of 2,2'-(ethylenedioxy)bisethylamine and 4,7,10-trioxa-1,3-tridecanediamine ~~of formula (I):~~

~~H2N–R–X (I);~~

~~wherein X is selected from the group consisting of NH2, OH, and CO2CH-2CH3; and~~

~~R is selected from the group consisting of an alkyl group and an alkyl ether group;~~

~~wherein, when X is NH2 or CO2CH2CH3, R comprises from 1 to 20 carbon atoms; and when X is OH, R comprises from 4 to 20 carbon atoms.~~

wherein the treatment results in the reaction of the succinimide ester groups with the amine functionality of the amine compound to form an amide linkage.

2. (canceled)

3. (canceled)

4. (original) The method of claim 1, wherein the ratio of equivalents of amine compound to equivalents of carboxylate groups is at least 50.

5. (canceled)

6. (canceled)

7. (original) The method of claim 1, wherein the aqueous mixture has a pH of at least 7.0.

8. (original) The method of claim 1, wherein the particles covalently bind less than 0.35 milligrams per square meter of non-specific protein when contacted with serum.

9. (canceled)

10. (canceled)

11. (canceled)

12. (canceled)

13. (original) The method of claim 1, wherein the particles physically adsorb less than 3 milligrams per square meter of non-specific protein when contacted with serum.

14. (canceled)

15. (canceled)

16. (currently amended) A sensitized particle for use in immunoassays, comprising:

a particle comprising a surface;

at least one antibody bound to the surface through a covalent bond from reaction of an N-hydroxysuccinimide or N-hydroxysulfosuccinimide/carbodiimide-activated carboxylate group on the particle surface with an amine group on the antibody; and

the reaction product of a succinimide ester and an amine compound on the surface selected from the group consisting of 2,2'-(ethylenedioxy)bisethylamine and 4,7,10-trioxa-1,3-tridecanediamine, ~~of formula (I) on the surface;~~

~~H2N–R–X— (I);~~

~~wherein X is selected from the group consisting of NH2, OH, and CO2CH2CH3; and~~

~~R is selected from the group consisting of an alkyl group and an alkyl ether group;~~

~~wherein, when X is NH2 or CO2CH2CH3, R comprises from 1 to 20 carbon atoms; and when X is OH, R comprises from 4 to 20 carbon atoms.~~

the reaction product being covalently attached to the particle surface.

17. (canceled)

18. (canceled)

19. (original) The sensitized particle of claim 16, further comprising BSA on the surface.

20. (original) The sensitized particle of claim 16, wherein the particle comprising a surface is selected from the group consisting of gold particles, ceramic particles, and polymer particles.

21. (original) The sensitized particle of claim 16, wherein the particles covalently bind less than 0.35 milligrams per square meter of non-specific protein when contacted with serum.

22. (canceled)

23. (canceled)

24. (canceled)

25. (canceled)

26. (original) The sensitized particle of claim 16, wherein the particles physically adsorb less than 3 milligrams per square meter of non-specific protein when contacted with serum.

27. (canceled)

28. (canceled)

29. (currently amended) A particle for use in immunoassays, comprising:

a polymer particle comprising a surface;

at least one antibody bound to the surface through a covalent bond from reaction of an N-hydroxysuccinimide or N-hydroxysulfosuccinimide/carbodiimide-activated carboxylate group on the particle surface with an amine group on the antibody;

BSA on the surface; and

the reaction product of a succinimide ester and an amine compound on the surface;

wherein the amine compound is selected from the group consisting of ~~glycine ethyl ester; 2-(aminoethoxy)ethanol;~~ 2,2'-(ethylenedioxy)bisethylamine[[;]] and 4,7,10-trioxa-1,3-tridecanediamine;

wherein the reaction product is covalently attached to the particle surface;

wherein the particles covalently bind less than 0.35 milligrams per square meter of non-specific protein when contacted with serum; and

wherein the particles physically adsorb less than 2 milligrams per square meter of non-specific protein when contacted with serum.

30. (currently amended) A reagent, comprising:

a plurality of particles;

each of said particles comprising a surface;

an antibody bound to the surface through a covalent bond from reaction of an N-hydroxysuccinimide or N-hydroxysulfosuccinimide/carbodiimide-activated carboxylate group on the particle surface with an amine group on the antibody; and

the reaction product of a succinimide ester and an amine compound on the surface selected from the group consisting of 2,2'-(ethylenedioxy)bisethylamine and 4,7,10-trioxa-1,3-tridecanediamine, ~~of formula (I) on the surface;~~

~~H2N-R-X (I);~~

~~wherein X is selected from the group consisting of NH2, OH, and CO2CH-2CH3; and~~

~~R is selected from the group consisting of an alkyl group and an alkyl ether group;~~

~~wherein, when X is NH2 or CO2CH2CH3, R comprises from 1 to 20 carbon atoms; and when X is OH , R comprises from 4 to 20 carbon atoms.~~

the reaction product being covalently attached to the particle surface.

31. (canceled)

32. (canceled)

33. (original) An assay method for determining an antigen, comprising:

combining a sample suspected of containing said antigen with the reagent of claim 30, the reagent comprising the antibody of said antigen, and the reagent capable of forming a detectable complex with said antigen; and

determining the presence or amount of said detectable complex as a measure of said antigen in said sample.

34. (original) A test kit, comprising the reagent of claim 30.

SPECIFICATION MARKED TO SHOW CHANGES

PARTICLES FOR IMMUNOASSAYS AND METHODS FOR TREATING THE SAME

This application is a continuation-in-part of co-pending U.S. Patent Application Serial No. [[__/_____]] 10/053,058, ~~attorney docket number 9793-76~~ filed November 2, 2001, entitled "Particles For Immunoassays And Methods For Treating The Same" filed November 2, 2001, with inventors C.C. Lawrence et al., which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

In testing for analytes such as drug molecules, immunoassays have proven to be especially useful. In an immunoassay, the interaction of an analyte, sometimes referred to as an antigen, with a specific receptor, typically an antibody, results in the formation of an antigen-antibody complex. This complex can be detected by various measurements, such as radioactivity, fluorescence, light absorption and light scattering. The results are then correlated with the presence, absence, and ideally the concentration of the analyte.

One type of particle-based agglutination immunoassay is based on the binding of an antigen with a particle-bound antibody. The particles employed are generally polymer particles, such as polystyrene and poly(methyl methacrylate), and are typically produced by an emulsion polymerization process. Other particle systems may also be used, including gold particles such as gold nanoparticles and gold colloids; and ceramic particles, such as silica, glass, and metal oxide particles. The antibody may be physically adsorbed onto the particle; however, greater stability and longer shelf-life are obtained when the antibody is covalently attached. See for example J.L. Ortega-Vinuesa et al. *J. Biomater. Sci. Polymer Edn.*, 12(4), 379-408 (2001).

Particles having covalently bound antibodies are typically prepared by activation of the particles, followed by coupling of antibodies to the activated particles. For particles having carboxylate groups bound to the surface, activation is often achieved by contacting the particles with a solution of a carbodiimide coupling reagent and a succinimide reagent such as N-

Immunoassay mixtures using these particles may also contain a tertiary amine compound to reduce interference due to the presence of tertiary amine groups on the particle surface. Suitable tertiary amine compounds include triethanolamine (TEO), as described in co-pending application serial no. [[__/_____]] 10/025,378, ~~attorney docket number 9793-74,~~ entitled "Tertiary Amine Compounds For Use In Immunoassays" filed December 18, 2001, with inventors C.C. Lawrence et al.

Particles of the present invention provide for a reduction in the amount of non-specific covalent binding of sample components. Figure 1 illustrates the characteristics of particles which have been activated and treated with the amine, but which have not been reacted with antibody. Specifically, Figure 1 is a graph of the amount of serum protein covalently bound to the particles as a function of the number of equivalents of the amine compound AEO added after the activation. Since no antibody is present, all proteins bound or adsorbed are non-specific proteins. Without amine treatment (zero equivalents of AEO), proteins from the serum mixture covalently bind to the activated particles, in this case providing a surface coverage of 1.5 milligrams per square meter (mg/m^2). Treatment with 200 equivalents of AEO, relative to the amount of carboxylate groups originally present, reduces the amount of covalently bound protein by about 85%.

The amount of non-specific protein covalently bound to particles is measured by the following test. A 750 microliter (µl) sample containing 0.5% (w/v) particles, 5% (v/v) normal human serum, and 50 mM 3-morpholino-propanesulfonic acid buffer (MOPS) at pH 7.0 is incubated at 37°C for 2 hr and centrifuged (15,000 x *g*, 30 min). The sample may not contain an antigen which will form a complex with any antibody bound to the particles. The particles are resuspended in 1 ml of 50 mM MOPS buffer at pH 7.0 and centrifuged again to remove excess, non-bound serum. This resuspension and centrifugation is repeated three more times. The resulting particles are resuspended by manual pipetting in 100 µl of a solution containing 6% (w/v) sodium dodecyl sulphate (SDS), 10% (v/v) glycerol and 60 mM Tris at pH 6.2, and are incubated at 80°C for 2 hr to effect desorption of non-covalently

The treatment of the particles with an amine compound of the present invention may be used alone or in combination with other techniques for reducing interference in an immunoassay. In optimizing the performance of particle agglutination immunoassays, it may be preferred to use sensitized particles which have been treated with a primary amine compound according to the present invention, and also to include a tertiary amine compound in the assay mixture as described in the above mentioned co-pending application serial no. [[__/_____]] 10/025,378, ~~attorney docket number 9793-74,~~ entitled "Tertiary Amine Compounds For Use In Immunoassays" filed December 18, 2001, with inventors C.C. Lawrence et al. In some cases, the use of a tertiary amine compound such as TEO in the immunoassay mixture may be sufficient to reduce the interference of the immunoassay to the desired level. The use of either the primary amine particle treatment or the tertiary amine compound additive, alone or in combination, can be determined empirically to determine if one technique is better than the other or if the combination yields the best results.

Various ancillary materials will frequently be employed in an assay in accordance with the present invention. For example, buffers will normally be present in the assay medium, as well as stabilizers for the assay medium and the assay components. Frequently, in addition to these additives, additional proteins may be included, such as albumin; or surfactants may be included, particularly non-ionic surfactants and the like.

The particles may, along with other reagents, be packaged in a kit useful for conveniently performing the assay methods for the determination of an analyte. To enhance the versatility of the subject invention, reagents can be provided in packaged combination, in the same or separate containers, in liquid or lyophilized form so that the ratio of the reagents provides for substantial optimization of the method and assay. The reagents may each be in separate containers, or various reagents can be combined in one or more containers depending on the cross-reactivity and stability of the reagents.

For example, a reagent test kit which may contain, in packaged combination, an antibody specific for a particular analyte, a particle of the